

Brief Report

Direct Interaction of Arabidopsis SGO1 with PP2A B' Subunits Revealed by Microscale Thermophoresis

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Abstract

Accurate chromosome segregation during meiosis requires protection of centromeric cohesion mediated by shugoshin (SGO) proteins and Protein Phosphatase 2A (PP2A). In *Arabidopsis thaliana*, genetic and cytological studies have demonstrated that PP2A B' regulatory subunits are essential for maintaining centromeric cohesion; however, direct biochemical evidence for their interaction with SGO1 in plants is lacking. Here, we used microscale thermophoresis (MST) to investigate and quantify the binding between fluorescently labeled GST-SGO1 and the PP2A B' α and B' β subunits. SGO1 exhibited concentration-dependent binding to both subunits, with dissociation constants (K_d) of $5.49 \pm 0.88 \mu\text{M}$ for B' α and $1.89 \pm 0.38 \mu\text{M}$ for B' β , indicating an approximately threefold higher affinity for B' β . These results provide direct quantitative biochemical evidence for a specific interaction between Arabidopsis SGO1 and PP2A B' subunits.

Keywords: shugoshin; SGO1; PP2A; B' subunit; centromeric cohesion; meiosis; microscale thermophoresis; *Arabidopsis thaliana*

1. Introduction

Faithful chromosome segregation in meiosis relies on the stepwise release of sister chromatid cohesion. Homologous chromosomes separate during meiosis I, whereas sister chromatids remain associated until meiosis II. This requires protection of centromeric cohesion until anaphase II, a process mediated by the conserved shugoshin (SGO)–Protein Phosphatase 2A (PP2A) module [1–3]. Shugoshin recruits PP2A to centromeres, where it counteracts phosphorylation of the meiosis-specific cohesin subunit REC8 (SYN1 in Arabidopsis), thereby protecting centromeric cohesion from separase-dependent cleavage during meiosis I [4,5].

PP2A is a heterotrimeric serine/threonine phosphatase composed of a scaffolding A subunit, a catalytic C subunit, and a regulatory B subunit that determines substrate specificity and localization [6,7]. In plants, the B subunit family is highly diverse and is classified into B, B', B'', and B''' subfamilies. The B' (B56) subfamily has undergone notable expansion in Arabidopsis, comprising 17 isoforms [7]. Members of the B' (B56) family play a central role in targeting PP2A to centromeric cohesion [1,2]. In yeast and animals, direct interaction between shugoshin and B' / B56 subunits has been characterized using structural and biochemical approaches [6,8–10]. Arabidopsis contains two shugoshin homologs, SGO1 and SGO2, of which SGO1 is the primary regulator responsible for protecting centromeric cohesion during meiosis. In Arabidopsis, genetic and cytological analyses have shown that PP2A B' α and B' β subunits are redundantly required for centromeric cohesion



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protection during meiosis, and that SGO1 is necessary for proper PP2A localization at centromeres [11–13]. However, direct biochemical evidence for the interaction between *Arabidopsis* SGO1 and these B' subunits is lacking. In this Brief Report, we address this gap using microscale thermophoresis (MST). In this study, we investigated whether *Arabidopsis* SGO1 directly interacts with the PP2A B' regulatory subunits B' α and B' β . Using microscale thermophoresis (MST), we provide direct quantitative evidence for specific binding between SGO1 and both PP2A B' subunits and determine their binding affinities. These findings provide biochemical support for the conserved SGO1–PP2A interaction in plants and contribute to a better understanding of the molecular basis of centromeric cohesion protection.

2. Materials and Methods

Full-length coding sequences of SGO1, PP2AB' α , and PP2AB' β were amplified from *Arabidopsis thaliana* cDNA and cloned into pGEX-6P-1 vectors for expression as GST fusion proteins. cDNA was synthesized from total RNA using SuperScript[®] III Reverse Transcriptase (Invitrogen, Carlsbad, CA, USA). The coding sequences of ATB' α (AT5G03470), ATB' β (AT3G09880), and SGO1 (AT3G10440) from *Arabidopsis thaliana* were amplified by PCR using gene-specific primers. PCR amplification was performed using the Expand[™] High Fidelity PCR System (Roche, Basel, Switzerland) with an initial denaturation at 94 °C for 2 min, followed by 35 cycles of 94 °C for 30 s, 60 °C for 30 s, and 72 °C for 1 min per kb, with a final extension at 72 °C for 7 min. ATB' α was amplified using the forward primer 5'-GAATTCATGTTTAAGAAGATCATGAAAGGG-3' and reverse primer 5'-CTCGAGCTAAGAAGTGATCATAGGATCTTC-3'. ATB' β was amplified using the forward primer 5'-GAATTCATGTTTAAGAAAATCATGAAAGGT-3' and reverse primer 5'-CTCGAGCTAGGAAGTGATCATATGATCTTC-3'. Because amplification of full-length SGO1 from flower-bud cDNA proved technically challenging, the coding sequence was initially amplified as two overlapping fragments using the primer pairs 5'-GAATTCATGGTTCGAGCGACGGTCTGAAT-3'/5'-CAACCACCTTTTGCAAGGT-TTCTG-3' and 5'-CAGAAACCTTGCAAAAGGTGGTTG-3'/5'-CTCGAGTCAGAAAGCC-CCTCGCATCTTCTC-3'. The two SGO1 fragments were subsequently assembled by overlap-extension PCR prior to cloning into the final expression construct.

Recombinant proteins were expressed in *Escherichia coli* BL21 Rosetta cells following induction with 0.1 mM IPTG at 20 °C and purified using glutathione–agarose affinity chromatography. Low-temperature induction conditions were selected to improve recovery and stability of recombinant full-length SGO1 protein during bacterial expression. Protein purity was assessed by SDS–PAGE.

For microscale thermophoresis, purified SGO1 was fluorescently labeled using NT-647 NHS dye according to the manufacturer's instructions (NanoTemper Technologies, Munich, Germany). Serial dilutions of PP2AB' α and PP2AB' β (200 μ M to 10 nM) were prepared in PBS and mixed 1:1 with 10 nM labeled SGO1. After 10 min incubation at room temperature, samples were loaded into premium-coated capillaries and analyzed using a Monolith NT.115 instrument. Binding curves were generated from three independent experiments and fitted using GraphPad Prism version 7.0 (GraphPad Software, San Diego, CA, USA) with a one-site binding model.

To examine the conservation of the shugoshin N-terminal coiled-coil region, protein sequence alignment of SGO1 proteins from different species was performed using Clustal Omega version 1.2.4 [14].

3. Results and Discussion

3.1. Interaction of SGO1 with PP2A B' Subunits

To determine whether Arabidopsis SGO1 directly interacts with PP2A B' regulatory subunits, we employed microscale thermophoresis (MST), a powerful, immobilization-free biophysical technique that enables the quantification of biomolecular interactions in solution with minimal sample consumption. Fluorescently labeled GST-SGO1 was titrated with increasing concentrations of purified GST-tagged PP2A B' α or GST-tagged PP2A B' β . Both subunits produced clear, concentration-dependent changes in thermophoretic mobility that approached saturation at higher ligand concentrations, consistent with specific and saturable binding interactions (Figure 1A,B). The resulting binding curves were well fitted to a one-site interaction model, yielding dissociation constants (K_d) of $5.49 \pm 0.88 \mu\text{M}$ for the B' α subunit and $1.89 \pm 0.38 \mu\text{M}$ for the B' β subunit. This indicates an approximately threefold higher binding affinity of SGO1 for the B' β subunit. A binding experiment between GST and fluorescently labelled recombinant purified GST-SGO1 was also performed as a negative control to rule out any non-specific interaction between SGO1 and the GST domain of the fusion constructs. No binding was observed between GST-SGO1 and GST alone (Figure 1C), confirming that the observed interactions with the PP2A B' regulatory subunits, PP2A B' α and PP2A B' β , were specific and not mediated by the GST tag. Successful purification of soluble full-length SGO1 furthermore enabled direct quantitative assessment of the interaction in the absence of additional plant-specific cellular components.

Microscale thermophoresis analysis of the interaction between SGO1 and PP2A B' subunits

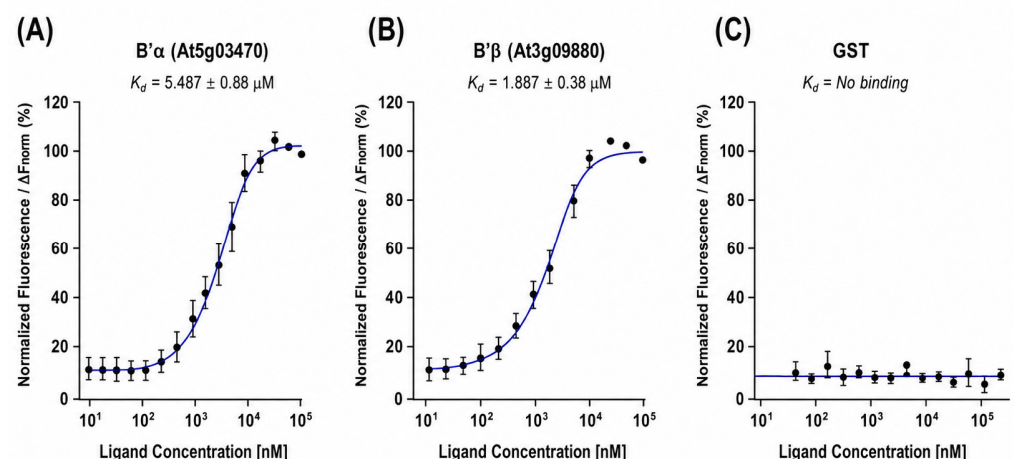


Figure 1. Microscale thermophoresis analysis of the interaction between SGO1 and PP2A B' subunits. Fluorescently labeled GST-SGO1 was mixed with increasing concentrations of (A) GST-PP2AB' α or (B) GST-PP2AB' β , resulting in concentration-dependent binding curves. (C) No interaction was detected between GST and SGO1.

These measurements provide the first quantitative biochemical characterization of the interaction between Arabidopsis SGO1 and PP2A B' subunits. The reproducible difference in affinity observed here between B' α and B' β represents a novel biochemical observation in plants and reveals a measurable difference in binding affinity between the two regulatory subunits.

Previous genetic and cytological studies have established that PP2A B' α and B' β subunits are redundantly required for protection of centromeric cohesion during meiosis in Arabidopsis, and that SGO1 is necessary for their proper localization to centromeres [11,12]. These biochemical observations are fully consistent with earlier genetic analyses of Arabidopsis pp2a b' α b' β mutants displaying defects in meiotic centromeric cohesion. Bimolec-

ular fluorescence complementation (BiFC) experiments further supported associations between SGO1 and these B' subunits [12].

However, those approaches were performed in non-meiotic somatic cell contexts (e.g., leaf protoplasts), relied on indirect readouts, and could not distinguish direct physical binding from indirect associations. In contrast, the present MST measurements, using highly purified recombinant proteins in a defined buffer system, provide direct biochemical evidence of physical interaction together with precise quantitative binding parameters. The ability of MST to measure interactions in solution without immobilization artifacts further strengthens the reliability of these affinities.

Sequence alignment of SGO1 proteins from *Arabidopsis thaliana*, *Homo sapiens*, *Oryza sativa*, and *Zea mays* showed that Arabidopsis SGO1, like its homologues, lacks the canonical LxxIxE short linear motif commonly recognized by B56/B' subunits [8,10]. The alignment also highlights the N-terminal region corresponding to the coiled-coil domain previously implicated in PP2A-B56/B' binding [6] (Figure S1). Instead, interaction is mediated through a conserved N-terminal coiled-coil domain that engages a hydrophobic pocket on the B' subunit surface [6]. Our quantitative MST data now provide direct biochemical validation that this motif-independent recruitment mechanism is functionally conserved in plants. These biochemical findings are consistent with previous genetic and cytological studies demonstrating the requirement of AtSGO1 for proper centromeric localization of B' α and B' β and the cohesion defects observed in *sgo1* mutants [11–13]. Thus, the AtSGO1–PP2A interaction is best interpreted not as a classical short-motif docking event, but as a structured recruitment mechanism. This interpretation explains how Arabidopsis SGO1 can lack a canonical LxxIxE motif while still directly binding PP2A B' subunits in vitro, and it provides a mechanistic framework for understanding the different affinities observed for B' α and B' β .

3.2. Structural Basis of the SGO1–PP2A Interaction

From an evolutionary perspective, shugoshin-mediated recruitment of PP2A appears to rely primarily on preservation of a structured N-terminal interaction interface rather than conservation of a short linear sequence motif. Comparative features of shugoshin–PP2A–B'/B56 interaction mechanisms across eukaryotes are summarized in Table S1. Arabidopsis SGO1 lacks the canonical LxxIxE motif commonly associated with B56/B' recognition, yet retains conserved hydrophobic and predicted coiled-coil features characteristic of shugoshin proteins from yeast and animals. This observation supports the idea that the underlying recruitment mechanism is conserved at the level of structural organization rather than strict primary-sequence identity. Such structured interfaces are thought to provide more stable and spatially organized interactions than classical short linear motif-mediated docking events. We also evaluated AlphaFold3 predictions [15] for the Arabidopsis SGO1–PP2A B' α and SGO1–PP2A B' β complexes. However, the predicted protein–protein interfaces showed low confidence scores, and the models were therefore not included in this study.

Within the expanded Arabidopsis B' family, B' α and B' β perform specialized functions in meiotic centromeric cohesion protection [11,12]. The approximately threefold higher affinity of SGO1 for B' β observed here demonstrates a measurable biochemical difference between the two subunits. Whether this difference contributes to functional specialization remains to be determined. Although the two subunits are genetically redundant at the organismal level, differential affinity may contribute to robustness of centromeric PP2A recruitment during meiotic progression. Collectively, these findings support a model in which the core shugoshin–PP2A regulatory architecture is evolutionarily conserved across

eukaryotes while lineage-specific diversification of PP2A regulatory subunits introduces additional layers of regulatory specialization (Figure 2).

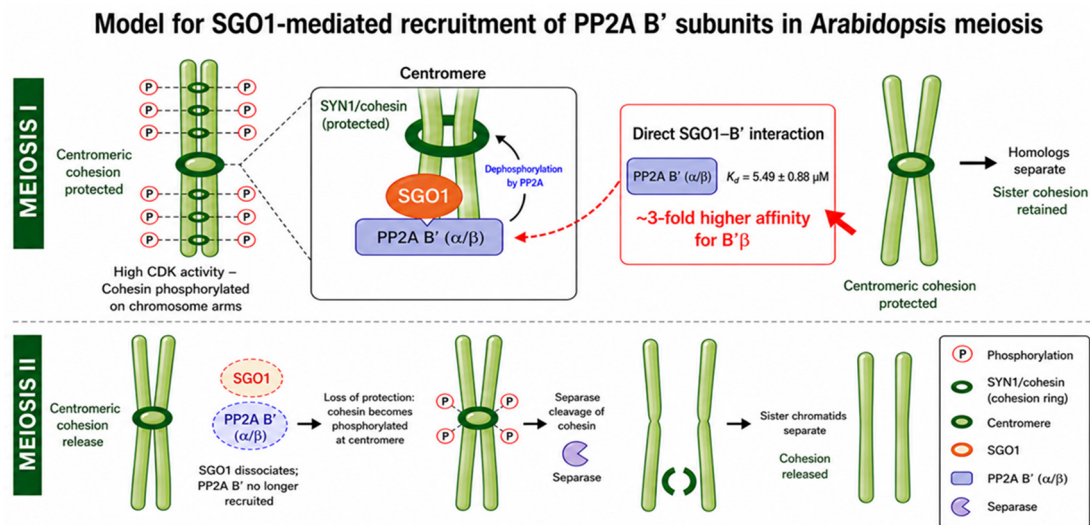


Figure 2. Proposed model for SGO1-mediated recruitment of PP2A B' subunits to protect centromeric cohesion in Arabidopsis meiosis. SGO1 directly interacts with both B'α and B'β subunits, with higher affinity for B'β. This interaction recruits PP2A to centromeres, where it dephosphorylates cohesin (SYN1) during meiosis I, protecting it from separase cleavage until meiosis II.

In conclusion, this study establishes a robust biochemical foundation for the SGO1–PP2A interaction. The quantified differences in binding affinity between B'α and B'β provide a basis for future structural and functional studies of PP2A recruitment.

Supplementary Materials: The following supporting information can be downloaded at <https://www.mdpi.com/article/10.3390/ijpb17070056/s1>, Figure S1. N-terminal sequence alignment of SGO1 proteins from *Arabidopsis thaliana* (AtSGO1), *Homo sapiens* (HsSGO1), *Oryza sativa* (OsSGO1), and *Zea mays* (ZmSGO1). Hydrophobic residues are highlighted in yellow. The bracket denotes the N-terminal coiled-coil region involved in PP2A-B56/B' binding. Table S1. Comparative features of shugoshin–PP2A-B'/B56 interaction mechanisms across eukaryotes.

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